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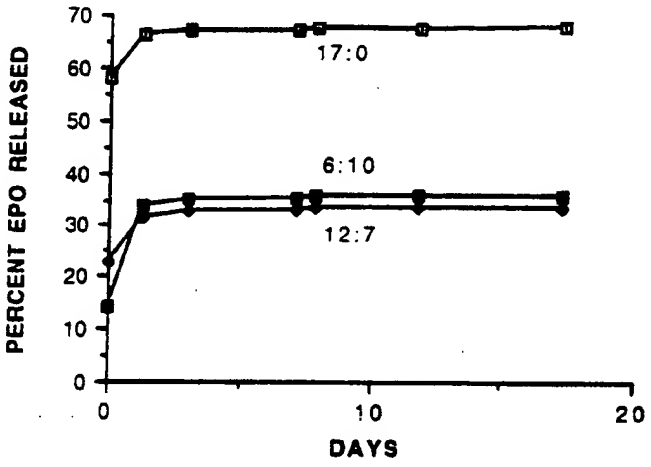

(57) Abstract

A method is described for the incorporation of proteins in the form of specific noncovalent complexes with polycationic reagents, into sustained release systems, where the polycation stabilizes the protein against inactivation while it resides in the delivery device, and retards release of the protein from the delivery device. Alternatively, the polycation-protein complex itself serves as a depot for release of the protein active agent, rather than a polymeric matrix. The end result is the release of the active agent with retention of biological activity, with a high cumulative field, over a sustained period of time.

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STABILIZATION OF PROTEINS BY CATIONIC BIOPOLYMERS

Background of the Invention

This invention is in the field of delivery systems for pharmaceutical agents and is especially related to methods for the stabilization of proteins using cationic polymers.

Sustained release devices have been developed over the past several years based on a broad range of technologies, directed to the delivery of a wide selection of pharmaceutical agents. The physical formats for such devices include use of microparticles, slabs or similar macroscopic systems designed for implantation, gels and emulsions, and other preparations conceived to preserve the active agent in the delivery system for an extended period of time.

The mechanism of release from matrix-type sustained release devices is generally understood to occur by hindered diffusion of the active agent through the carrier matrix, or by erosion of the matrix over time resulting in the liberation of the incorporated active agent. These processes are not mutually exclusive, and both mechanisms may be simultaneously active in the case of a given system.

In recent years sustained release devices have been used for the delivery of protein pharmaceutical agents, primarily as a result of the availability of recombinant proteins which have been developed for therapeutic applications in a wide variety of pathological conditions. Development of such systems creates greater challenges to overcome than in the case of low molecular weight drugs and pharmaceutically active substances, since proteins inherently have only marginal conformational stability, and can frequently be susceptible to conditions or processes which result in inactivation or denaturation. In contrast to the degradation or deterioration of low molecular weight pharmaceuticals, the structural alterations in proteins leading to inactivation need not involve changes in the covalent structure of the protein, but can be entirely the consequence

of disruption of an extensive system of noncovalent interactions which are responsible for the preservation of the native three dimensional structure of the protein. This is the basis for the greater lability of proteins.

Certain features of sustained release devices exacerbate the potential for the inactivation of protein active agents. These include the fact that large amounts of solid protein are introduced into the delivery system (either as pure preparations or mixed with additives and excipients), and that the physical attributes of the delivery systems themselves may present interfaces which promote denaturation. Hydrating the solid protein under physiological conditions *in vivo* results in formation of a protein gel or a highly concentrated solution of the protein. Under these circumstances it is quite possible for the protein to become aggregated or denatured due to interactions with neighboring molecules or upon exposure to the interface with the delivery system.

In order to overcome these potential problems, proteins have been formulated with excipients intended to stabilize the protein in the milieu of the pharmaceutical product. It has long been known that a variety of low molecular weight compounds have the effect of preserving the activity of proteins and enzymes in solution. These include simple salts, as described by P. H. von Hippel and K.-Y. Wong, "Neutral Salts: the Generality of Their Effects on the Stability of Macromolecular Conformations", Science 145, 577-580 (1964), buffer salts and polyhydroxylated compounds such as glycerol, mannitol, sucrose and polyethylene glycols, K. Gekko and S. N. Timasheff, "Mechanism of Protein Stabilization by Glycerol: Preferential Hydration in Glycerol-Water Mixtures", Biochemistry 20, 4667-4676 (1981); K. Gekko and T. Morikawa, "Preferential Hydration of Bovine Serum Albumin in Polyhydric Alcohol-Water Mixtures", J. Biochem. 90, 39-50 (1981); and J. C. Lee and L. L. Y. Lee, "Preferential Solvent Interactions between Proteins and Polyethylene Glycols", J. Biol. Chem.

256, 625-631 (1981). Certain biocompatible polymers have also been applied for this purpose, such as various polysaccharides and synthetic polymers including polyvinylpyrrolidone, for example. Even benign detergents such as polyoxyethylene sorbitan monooleate (Tween 80™) have been included to preserve bioactivity in pharmaceutical formulations. Use of these materials has been implemented over many years, for example, with soluble preparations of vaccines and insulin, long before recombinant protein pharmaceutical agents became available.

Except for the detergents, the mechanism by which these substances exert their stabilizing effect has become evident in recent years as a result of thorough investigation. It has been shown that stabilization occurs as a result of a general thermodynamic phenomenon prevalent in these ternary systems, wherein the cosolute (for example, the polyol) is preferentially excluded from the domain of the protein, and the protein is preferentially hydrated. As a result, the protein is stabilized by enhancement of the hydrophobic interactions which are generally thought to confer stability on the native tertiary structure of the protein, as compared with the protein in the absence of the cosolute.

Use of these excipients may be associated with certain disadvantages. For example, the thermodynamic effects require high concentrations of the cosolute in order to be effective. Under certain conditions, high concentrations of polysaccharides may even lead to phase separation of the protein. Alternatively, low molecular weight excipients have high solubilities and high diffusion coefficients, so that they are depleted from the delivery device considerably more rapidly than the active agent. The beneficial effects of the excipient are therefore transient, occurring only in the initial stages of the duration of the release of the protein. This condition leaves the protein pharmaceutical still within the

sustained release device, prone to inactivation due to intermolecular aggregation and interaction with the surface of the device.

It is therefore an object of the present invention to enhance the amount of release and stability of proteins incorporated into polymeric matrices for controlled drug delivery.

It is a further object of the present invention to provide a method and compositions that can be used with a variety of compounds to enhance stability, with minimum effort and expense.

It is another object of the present invention to provide a method and compositions that can be used as biodegradable, biocompatible depots for controlled drug delivery.

Summary of the Invention

A method is described for the incorporation of biologically active agents, especially protein pharmaceutical agents, in the form of specific noncovalent complexes with polycationic reagents, into sustained release systems, where the polycation stabilizes the protein against inactivation while it resides in the delivery device, and retards release of the protein due to the added effects of dissociation of the complex according to the law of mass action. The end result is the release of the active agent with retention of biological activity, with a high cumulative yield, over a sustained period of time.

In a second embodiment of this method and compositions, the polycation-protein complex itself serves as a depot for release of the protein active agent, rather than a polymeric matrix. In the most preferred embodiment, the complexing polyelectrolyte is both biocompatible and biodegradable.

Examples are provided demonstrating complex formation (for example, between erythropoietin and chitosan) and enhanced stability and release from polymeric devices of proteins (such as Factor VIII).

Brief Description of the Drawings

Figure 1 is the percent erythropoietin (EPO) released from poly(DL-lactide-co-glycolide) (50:50) microspheres in 50 mM sodium phosphate pH 7.3 at 37°C, for EPO:chitosan ratios, expressed as percents of total solids, of 12:7, 6:10 and 17:0 over time (days).

Figure 2 is a graph of the cumulative units of Factor VIII activity released per mg of poly(lactic acid) microspheres, containing either poly(arginine) (2 mg/ml) complexed with Factor VIII at 30% loading, over time (days) or Factor VIII in NaCl-$CaCl_2$-glycine buffer.

Figure 3 is a graph of the percent cumulative release over time (days) for bovine serum albumin (BSA):sucrose (5:5) (light squares); BSA:protamine (5:5) (triangles); and BSA (dark squares), all at 10% by weight loading.

Detailed Description of the Invention

The majority of the prior art processes and phenomena relating to stability and release of compounds from polymeric matrices is based on general physical chemical principles, except for the process of erosion of sustained release systems, which involves actual chemical degradation of the matrix. The method and compositions described herein, in contrast, are based on a reversible chemical interaction between the compound to be released and a stabilizing compound.

In the preferred embodiment, the biologically active agent is a protein or peptide (including natural, recombinant, synthetic, high and low molecular weight pr teins or peptides). It could also be a nucleic acid, a

polysaccharide, a carbohydrate or derivatives thereof, a low molecular weight organic molecule or pharmacological agent. Complex formation between proteins and biological polycations can be used for proteins whose isoelectric point (pI) is acidic or neutral, as well as any protein having acidic side chains clustered together on the surface of the protein when it is in its native, active conformation. Proteins with acidic or neutral pI values have a preponderance of acidic over basic side chains in their structures. These are the groups which are available for interaction with the polycation, primarily by electrostatic interactions. The polycation has the capability of binding several molecules of protein per molecule of polycation. If the protein is also polyvalent in binding sites for the polycation, the complex will likely aggregate or precipitate, in analogy to the antigen-antibody precipitin reaction. If the protein is monovalent for the polycation the complex will remain soluble, presumably as a complex comprised of many protein molecules bound to each polycation molecule. The complexed protein is stabilized relative to the case of the absence of the polycation, both in aqueous solution or suspension, and when incorporated into sustained release devices.

The polycation must be biocompatible and, preferably, biodegradable. A variety of polycations can be used. Simple polyamino acids such as poly(lysine) or poly(arginine) are useful materials. Their molecular weights should be 4,000 daltons or greater, preferably about 50,000 or greater. Protamine is another useful polycation. Chitosan is useful primarily for acidic proteins, since it precipitates at pH values greater than about 6.5. Other biological polycations are also applicable for the purposes of this invention.

The weight ratio of protein to polycation can be in the range 1:1000 (when the protein has a very high biological activity per unit weight, so that the overall dosing requirement is low) to 20:1 (in the

converse situation). The preferred range for the weight ratio will be 1:100 to 10:1. The pH at which the complex is formed will affect the process. The overall state of charge of the protein will be a function of pH, since proteins are polyampholytes. The pH must be one at which the protein retains full biological activity, which is a property unique to each protein. The pH may also affect the charge on the polycation in certain cases, or, as with chitosan, actually affect its solubility. Of course, once introduced *in vivo*, release devices incorporating these complexes will experience pH values approximating physiological pH.

The fabrication of sustained release systems containing protein-polycation complexes differs little from the processes currently used for incorporating protein formulations. Liquid formulations can be employed in the manufacture of sustained release microspheres in conventional solvent evaporation procedures. Solid formulations, typically prepared as lyophilized solids from the liquid, can also be used. In particular, solid preparations of protein-polycation formulations can be micronized, i.e., fragmented to produce particles in the size range from less than 1 micrometer to about 5 micrometers, using the procedures outlined by Gombotz, et al., in U.S. Serial No. 07/345,684 filed May 1, 1989, the teachings of which are incorporated herein, summarized as follows.

The biologically active molecule is first dissolved in a solvent that can be lyophilized to form a solution having a concentration ranging from approximately 0.1 to 25% (w/v). The solvent may be pure water or can be buffered to a particular pH or ionic strength. The solvent may also be organic. The solution may contain the biologically active molecule alone, mixtures of two or more types of biologically active molecules alone, mixtures of biologically active molecules and stabilizers, or any combination thereof. In order to reduce the particle size of these preparations to the greatest extent, the composition should be suspended in

a medium in which not only the solvent but also the buffer salts are volatile under conditions of lyophilization. Examples of buffers removed by lyophilization include ammonium bicarbonate and other volatile ammonium salts.

The solution is then atomized into a low temperature liquified gas using any one of several devices, such as ultrasonic nozzles, pressure nozzles, pneumatic nozzles and rotary nozzles. The liquified gas can be liquid argon (-185.6°C), liquid nitrogen (-195.8°C), liquid oxygen (-182.9°C) or any other gas that results in the immediate freezing of the atomized particles into frozen particles. Oxygen is not preferred for proteins since it is explosive and may also cause oxidation of the protein.

The liquified gas is removed by evaporation at a temperature at which the solvent remains frozen, leaving behind frozen particles. The frozen solvent is removed from the particles by lyophilization to yield porous particles. These particles can vary in diameter depending on the technique used for their atomization, but generally range from approximately 10 to 50 micrometers.

These protein particles can be incorporated into biodegradable polymer microspheres using the processes taught by Gombotz, et al., U.S. Serial No. 07/346,143 filed May 1, 1989, the teachings of which are incorporated herein, or other more conventional techniques. Polymers that can be used to form the microspheres include bioerodible polymers such as poly(lactic acid), poly(lactic-co-glycolic acid), poly(caprolactone), polycarbonates, polyamides, polyanhydrides, polyamino acids, polyortho esters, polyacetals, polycyanoacrylates and degradable polyurethanes,and non-erodible polymers such as polyacrylates, ethylene-vinyl acetate and other acyl substituted cellulose acetates and derivatives thereof, non-erodible polyurethanes, polystyrenes, polyvinyl chloride, polyvinyl

fluoride, poly(vinyl imidazole), chlorosulphonated polyolefins, and polyethylene oxide.

The method of Gombotz, et al., is summarized as follows. Polymer and agent to be encapsulated in solution or dispersion are atomized using an ultrasonic device into a liquified gas which overlays a bed of frozen non-solvent. The microspheres are immediately frozen by the liquified gas. The solvent is slowly removed from these spheres as they thaw and sink onto and then into very cold non-solvent which extracts the solvent as it and the spheres thaw, leaving microspheres containing the encapsulated agent. The liquified gas can be liquid argon (-185.6°C), liquid nitrogen (-195.8°C), liquid oxygen (-182.9°C) or any other gas that results in the immediate freezing of the atomized particles into frozen spheres.

The product microspheres have been shown to exhibit sustained release *in vitro* and *in vivo* with a broad variety of proteins and enzymes.

The loadings of the active formulation of the protein-polycation complex in such sustained release systems can be from 5 to 50% (w/w), preferably in the range 10-40%.

Release of the protein active agent from microspheres containing protein-polycation complexes can occur according to one of several mechanisms. First, dissociation of the protein from the complex would occur only *in situ* in the domain of the sustained delivery system. The free protein diffuses out of the device, while the polycation remains behind. The polycation presumably is still bound in a network of the protein-polycation complex (in the case of proteins that are polyvalent for the polycation), or bound to other protein molecules (in the case of proteins that are monovalent for the polycation). In either case, it is likely that the diffusion coefficient of the polycation molecule is much lower than that of the free protein, so that it remains within the device. Second, the

protein-polycation complex, to the extent that it is soluble, diffuses out of the sustained delivery device into the release sink. It then undergoes dissociation to release the protein active agent into the medium. Third, free (i.e., uncomplexed) molecules of protein and polycation leave the sustained release device independently and possibly simultaneously. They remain uncomplexed to the extent permitted by the law of mass action. In reality, it is likely that a combination of these effects is operative.

It has been discovered that proteins can form complexes with biological polycations *in vitro*; in many cases turbidity or formation of a precipitate actually occurs. This observation has led to the use of such complexes as depots or reservoirs for stabilization of the protein active agent and for incorporation into sustained release systems. In this embodiment of this method and compositions, the polycation-protein complex itself serves as a depot for release of the protein active agent, rather than a polymeric matrix.

The requirements for a polycation-protein complex to serve as a reservoir for the sustained release of the protein as the active agent in a pharmaceutical formulation can be summarized as follows. First, the association constant for the formation of the complex should be relatively high, a property which may be achieved by virtue of cooperativity in the process of forming the complex. A consequence of having a high association constant is that the concentration of free protein will remain relatively low. Under such conditions, when the release mechanism is governed by diffusion, the rate of release can be diminished because the flux is proportional to the concentration gradient established between the inner and outer phases. With a low concentration of protein established in the inner phase, the rate of diffusion will be low. Second, the concentration of polycation should be relatively low, so that the active agent is the prevalent component by weight in the formulation, if so

desired. This is readily achievable because the high association constant ensures that most or all of the polycation participates in complex formation.

Third, the molecular weight of the polycation should be relatively high, so that its diffusion coefficient will be low. In this way the active agent will be preferentially depleted from the matrix or depot prior to the polycation.

The present invention will be further understood by the following non-limiting examples.

Example 1: Formation of a complex between bovine serum albumin and chitosan.

1 g of chitosan was dissolved in 100 ml of 1% acetic acid. The pH of the resulting solution was 3.0. The solution was titrated with sodium hydroxide to pH of 6.0, avoiding precipitation and gel formation by the chitosan. This is termed neutralized chitosan.

12.0 mg of bovine serum albumin (BSA) was dissolved in 1.0 ml 5 mM ammonium bicarbonate. 20 microliter aliquots of neutralized chitosan were added to the BSA, as well as to a buffer blank. A thick cloudy precipitate formed with the BSA, which was more profound and extensive than that observed with buffer alone. The latter is ascribed to pH-induced precipitation of chitosan. Centrifugation was used to determine whether precipitation occurred in the liquid supernatant with successive additions of chitosan. Generation of incremental turbidity ended at about the point where 200 microliters of the chitosan solution had been added to the BSA, corresponding to 2.0 mg chitosan.

The equivalence point was reached at a weight ratio of BSA:chitosan of about 5:1.

Example 2: Formation of a complex between bovine hemoglobin and chitosan.

10.2 mg of bovine hemoglobin (Hb) was dissolved in 1.0 ml deionized water. Up to 40 microliters of neutralized chitosan was added in portions. With the first additions a dark agglomerate formed, corresponding to partial depletion of color from the solution. Further addition of chitosan did not lead to a quantitative precipitation of the Hb.

Example 3: Preparation of and *in vitro* release from PLGA microspheres containing the erythropoietin-chitosan complex.

Chitosan acetate at pH 5 was used to dissolve recombinant human erythropoietin (EPO) with varying ratios of chitosan:EPO. These formulations were micronized according to the method set forth in Gombotz et al. (U. S. Serial No. 07/345,684) and incorporated into copoly(D,L-lactide, glycolide) (50:50, Boehringer-Ingelheim RG 503) using the procedures of Gombotz et al. (U. S. Serial No. 07/345,143). The final loading ratios, in weight percentages of the final microsphere preparation, were 6% EPO: 10.3% chitosan, 12% EPO: 6.8% chitosan, and 17% EPO alone.

These microspheres were subjected to *in vitro* release studies at 37°C, using the following release buffer: 50 mM sodium phosphate, 0.9% NaCl, 2% (w/v) ovalbumin, pH 7.2. The release results are shown in Figure 1. It is evident that, as compared to the absence of chitosan, incorporation of the polycation profoundly reduces the burst effect upon the release of EPO from the microspheres.

Example 4: Preparation and *in vitro* release of PLA microspheres containing the Factor VIII-poly(arginine) complex.

Human recombinant Factor VIII was reconstituted to 200 units/ml in 0.2 M NaCl, 0.55 M glycine, 0.005 M $CaCl_2$, 12 mg/ml human serum

albumin. To this solution was added polyarginine [$(Arg)_n$] at 2 mg/ml. The solution was subjected to a change in composition of the buffer to 0.1 M proline, 2.5 mM $CaCl_2$, pH 7.35 by passing the reconstituted mixture through a Sephadex® G-25 column equilibrated with the proline - $CaCl_2$ solution. The product was then micronized according to the procedure of Gombotz, et al., in U.S. Serial No. 07/345,684, and incorporated into microspheres comprised of poly(lactic acid) as the carrier matrix, at a loading of the formulated Factor VIII preparation of 30% (w/w) using the procedure described by Gombotz, et al. in U.S. Serial No. 07/345,143. This preparation is referred to as "poly(arginine)" in Figure 2.

A similar microsphere preparation was made using human recombinant Factor VIII reconstituted to 100 units/ml in 0.1 M NaCl, 0.275 M glycine, 0.0025 M $CaCl_2$, 6 mg/ml human serum albumin. This preparation was similarly incorporated into PLA microspheres at 30% loading. This preparation is referred to as "NaCl-Glycine" in Figure 2.

The two microsphere preparations were subjected to *in vitro* release experiments at 37°C, by immersing approximately 10 mg of microspheres in 1.0 ml aliquots of a release buffer consisting of 0.1 M NaCl, 0.1 M glycine, 10 mM HEPES, 2.5 mM $CaCl_2$, 2 mg/ml human serum albumin, pH 7.2 in a 1.5 ml microfuge tube, and agitated gently. Fresh aliquots of release medium were applied for each time point. The activity was assayed using the Coatest® kit for Factor VIII produced by Kabi Vitrum and distributed by Helena Laboratories, Inc. The color resulting from release of p-nitrophenolate from a synthetic substrate, as determined in microtiter plate format using a plate reader, and expressed as the cumulative percent of incorporated activity released per mg of microspheres, is given for the two preparations in Figure 2. It is evident that Factor VIII formulated with (Arg)n has led to markedly enhanced and sustained release kinetics compared to omission of (Arg)n.

Example 5: Bovine Serum Albumin-Protamine Complex Release from Copoly(lactide-glycolide) Microspheres.

A globulin-free preparation of bovine serum albumin (BSA) obtained from Sigma Chemical Co. was mixed 1:1 (w:w) with sucrose or with protamine sulfate. The resulting solutions, as well as a solution of BSA alone, were micronized according to Gombotz, et al., as described in U.S. Serial No. 07/345,684. The protein-excipient formulations were incorporated into microspheres of copoly(DL-lactide,glycolide) (50:50) following the procedures of Gombotz, et al., U.S. Serial No. 07/345,143, with total loadings of 10% by weight. These microspheres were placed in 20 mM sodium phosphate, 0.15 M sodium chloride, 1.5 mM sodium azide, pH 7.5, at 37 C to measure *in vitro* release.

The cumulative release over 68 days is shown in Figure 3. The surge in release that occurs between about days 20 and 28 is ascribed to degradation of the polymer matrix, exposing fresh reservoirs of protein for release to the medium. The results show that incorporation of protamine sulfate gives enhanced release characteristics as compared to the incorporation of an equal amount of sucrose. The extent of release in the first hour, termed the burst, is diminished, and the steady, near-zero-order release of protein is sustained for a longer duration. For BSA without added excipients, the burst release is the lowest of the three cases shown, but the degradation phase releases a large fraction of the protein over a relatively short period of time; further release continues for the remainder of the time period considered. Of the three preparations shown, the incorporation of protamine sulfate leads to the most monotonic release of protein after the burst.

We claim:

1. A stabilized composition for controlled release of a biologically active protein comprising

a biocompatible polymeric matrix incorporating a complex of a protein or peptide and a biocompatible polycation.

2. The composition of claim 1 wherein the polycation is complexed with the protein in a ratio between approximately 1:1000 protein:polycation by weight and 20:1 protein:polycation.

3. The composition of claim 1 wherein the polycation is complexed with the protein in a ratio between approximately 1:100 protein:polycation by weight and 10:1 protein:polycation.

4. The composition of claim 1 wherein the protein has a pI of less than 8.

5. The composition of claim 1 wherein the polymer forming the matrix is selected from the group consisting of biocompatible synthetic and natural polymers.

6. The composition of claim 5 wherein the polymer is selected from the group consisting of poly(lactic acid), poly(lactic-co-glycolic acid), poly(caprolactone), polycarbonates, polyamides, polyanhydrides, polyamino acids, polyortho esters, polyacetals, polycyanoacrylates and degradable polyurethanes, and non-erodible polymers such as polyacrylates, ethylene-vinyl acetate and other acyl substituted cellulose acetates and derivatives thereof, non-erodible polyurethanes, polystyrenes, polyvinyl chloride, polyvinyl fluoride, poly(vinyl imidazole), chlorosulphonated polyolefins, and polyethylene oxide.

7. The composition of claim 1 wherein the polycation is selected from the group of polyamino acids, basic proteins and cationic polysaccharides.

8. The composition of claim 1 wherein the polycation has a molecular weight of 4,000 daltons or greater.

9. The composition of claim 8 wherein the polycation has a molecular weight of about 50,000 or greater.

10. The composition of claim 1 wherein the matrix is the supramolecular aggregate formed by the polycation in complexed with the biologically active agent.

11. The composition of claim 10 wherein the protein is polyvalent in its interaction with the polycation.

12. A method for stabilizing a biologically active protein in a controlled release device comprising

forming a complex of a protein and biocompatible polycation and incorporating the complex into a biocompatible polymeric matrix.

13. The method of claim 12 wherein the polycation is complexed with the protein in a ratio between approximately 1:1000 protein:polycation by weight and 20:1 protein:polycation.

14. The method of claim 12 wherein the polycation is complexed with the protein in a ratio between approximately 1:100 protein:polycation by weight and 10:1 protein:polycation.

15. The method of claim 12 wherein the protein has a pI of less than 8.

16. The method of claim 12 wherein the polymer forming the matrix is selected from the group consisting of synthetic and natural polymers.

17. The method of claim 16 wherein the polymer is selected from the group consisting of poly(lactic acid), poly(lactic-co-glycolic acid), poly(caprolactone), polycarbonates, polyamides, polyanhydrides, polyamino acids, polyortho esters, polyacetals, polycyanoacrylates and degradable polyurethanes,and non-erodible polymers such as polyacrylates, ethylene-vinyl acetate and other acyl substituted cellulose acetates and derivatives thereof, non-erodible polyurethanes, polystyrenes, polyvinyl chloride, polyvinyl fluoride, poly(vinyl imidazole), chlorosulphonated polyolefins, and polyethylene oxide.

18. The method of claim 12 wherein the polycation is selected from the group of polyamino acids, basic proteins, and cationic polysaccharides.

19. The method of claim 12 wherein the polycation has a molecular weight of 4,000 daltons or greater.

20. The method of claim 19 wherein the polycation has a molecular weight of about 50,000 or greater.

21. The method of claim 12 wherein the polymeric matrix is formed by the polycation in combination with the protein.

22. The method of claim 16 wherein the protein is a protein polyvalent in its interaction with the polycation.

23. The method of claim 12 further comprising providing an amount of polycation in combination with protein effective to alter the release of the biologically active agent from the polymeric matrix.

24. The method of claim 12 further comprising providing an amount of polycation in combination with protein effective to maintain the activity of the protein as compared to the activity of the protein in the polymeric matrix in the absence of the polycation.

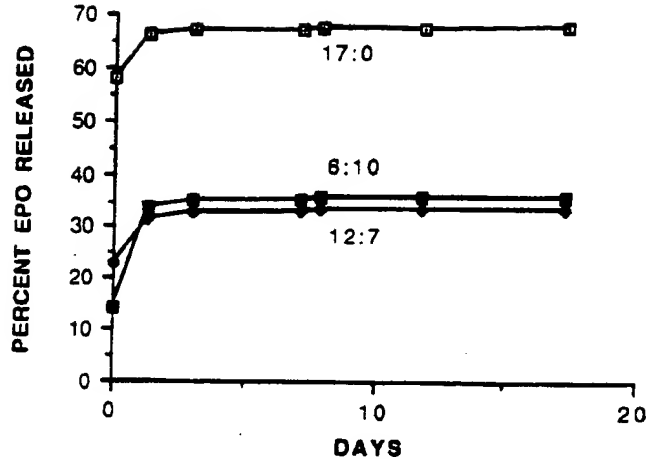

FIGURE 1

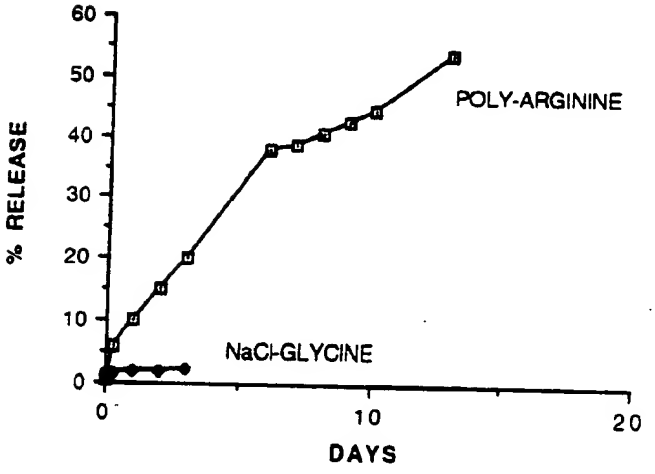

FIGURE 2

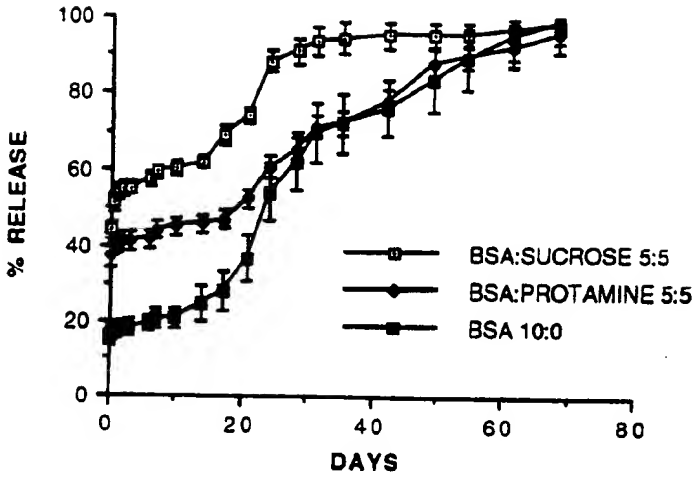
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FIGURE 3

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US91/09771

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC (5): A61K 9/26

U.S. CL. 424/484

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
U.S.	424/484, 486, 487, 488

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
Y	US, A, 4,883,669 (CHIEN ET AL) 28 NOVEMBER 1989 See entire document.	1-24
Y	US, A, 4,931,287 (BAE ET AL) 05 JUNE 1990 See entire document.	1-24
Y	US, A, 4,931,288 (EMBREY ET AL) 05 JUNE 1990 See entire document.	1-24

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
02 MARCH 1992	18MAR 1992
International Searching Authority	**Signature of Authorized Officer**
ISA/US	Thurman K. Page